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July 2, 1999

To: Docket Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Rm. 1061
Rockville, Maryland 20852

From: Haejung An and Paul Reno

Subject: Parameter Identification for a Risk Assessment for *Vibrio parahaemolyticus*
in Raw Molluscan Shellfish
Docket No. 99N-1075

We would like to take this opportunity to emphasize the potential for urease-negative strains of *Vibrio parahaemolyticus* (Vp) as virulent human pathogens. Strains with this phenotype have been isolated from patients exhibiting illnesses associated with consumption of contaminated shellfish during the last two years in the Northwest region of the U.S. On p. 11 of your document, the importance of urease was mentioned with the statement that "The presence of urease gene may provide a competitive environment advantage allowing access to a wider range of nutrients". It is our contention that the presence of this enzyme may also confer enhanced virulence to the pathogen when resident in the human gut after ingestion. Recent data supports this contention.

Compiled research data show that gastroenteritis in the Pacific Northwest is mainly associated with a urease-positive, Kanagawa-negative biotype of Vp (Kelly and Stroh, 1988 and 1989). Until recently, Kanagawa-positive strains have been recognized in other geographic locales as the primary virulent strains associated with Vp-associated disease. However, Kelly and Stroh (1989) reported that all the strains associated with disease caused by the ingestion of contaminated shellfish in the Pacific Northwest were urease positive, while all the strains associated with traveler's diarrhea were urease negative. Kelly and Stroh (1988) failed to confirm the previous results reported from Japan, India and Indonesia that the clinical isolates of Vp are Kanagawa hemolysin positive. The investigators reported that, although Kanagawa hemolysin-positive strains represented 1% of the environmental isolates of Vp in the Pacific Northwest (Sakazaki

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et al., 1968; Janda et al, 1988), clinical isolates from the human subjects with gastroenteritis were mostly urease-positive and Kanagawa-negative strains (Kelly and Stroh, 1989). The patients did not have a record of traveling out of the area during the probably incubation time for the disease, and thus the infecting Vp were most likely acquired locally in the Pacific Northwest region.

In comparison, the VP strains associated with diarrhea acquired while individuals were traveling in tropical areas, i.e., Thailand, Jamaica, and Florida, were Kanagawa hemolysin-positive. The importance of ureahydrolysis as virulent factor was also reported by Nolan et al. (1985). In 1981, six cases of gastroenteritis due to Vp infection were reported and the isolates from the five patients showed positive reaction for urea hydrolysis but in this case the isolates were also Kanagawa-positive. Recently, the important role of urease contributing to pathogenicity of Vp is well illustrated by Cai and Ni (1996). When urease produced by a clinical isolate of Vp was purified and tested by injection into suckling mice, the purified urease alone caused as much intestinal fluid accumulation as did Vp and its culture supernatant. This indicated that the strains of Vp producing this enzyme might have an enhanced ability to cause the signs of gastroenteritis seen in clinical cases of shellfish-borne disease. The significant increase in the proportion of urease-positive isolates in clinical disease cases compared to the much lower proportion of these biotypes in the environment could be a result either of an increased survival or growth potential in shellfish, or enhanced survival or growth in humans after ingestion, or both of these mechanisms. Consequently, it may be that urease confers a selective advantage in the "primary" host, shellfish or in the "secondary" host, humans. Which mechanism(s) is operative bears close study.

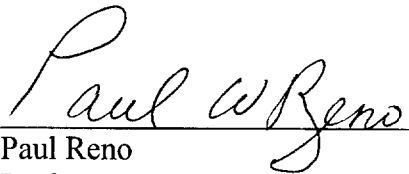
On p. 17, it is stated that "the incidence of pathogenic strains among natural populations of Vp in oysters is low and sporadic...." This statement probably does not reflect the prevalence of virulent strains of Vp in the Pacific Northwest, a region with significant shellfish harvest. It has been reported that urease-positive isolates comprise 58.4% of the total Vp in Willapa Bay, Washington (Kaysner et al., 1990). The environment research conducted later in the same study area showed a total of 57.2% of the isolates obtained from Grays Harbor and 28.5% of the Puget Sound isolates were urease-positive. Kaysner et al. (1994) reported that virulent urease-positive strains are now the predominant biotype of Vp IN the environment, as well as the predominant phenotype associated with gastroenteritis. The investigators suggested that the high percentage of urease-positive isolates was due to the spread or increased numbers of the urease-positive biotype conferred by some selective advantage of the presence of the enzyme, urease. This may reflect a phenomenon that is currently operative in the Pacific Northwest, since in comparison, urease-positive strains were found in low rates even in clinical isolates from patients with gastroenteritis in other countries: only 8% in Thailand (Suthienkul et al. 1995) and 6% in Japan (Osawa et al., 1996). It has been reported by the Oregon Department of Agriculture, Shellfish Monitoring Program, that two cases of Vp occurred in Oregon despite the fact all the oysters from the purported source of contamination had been monitored and fewer than 30 cells/g oysters (Cannon, 1999). Thus, it is apparent that the current high level of Vp which renders oysters unfit

for consumption may be higher than is safe for ingestion, especially if the Vp associated with the oysters have efficient virulence factors such as urease.

Thus, we would like to petition FDA to include urease-positive strains in the proposed risk assessment of Vp for shellfish. These biotypes may have enhanced virulence in humans rather than being simply more competitive in their natural estuarine/marine environment or in shellfish. As stated above, the occurrence of urease-positive strains is considerably elevated in the Pacific Northwest compared to Kanagawa-positive strains reported from other regions, and this may be relevant to the epidemics of gastroenteritis in this region associated with shellfish consumption.



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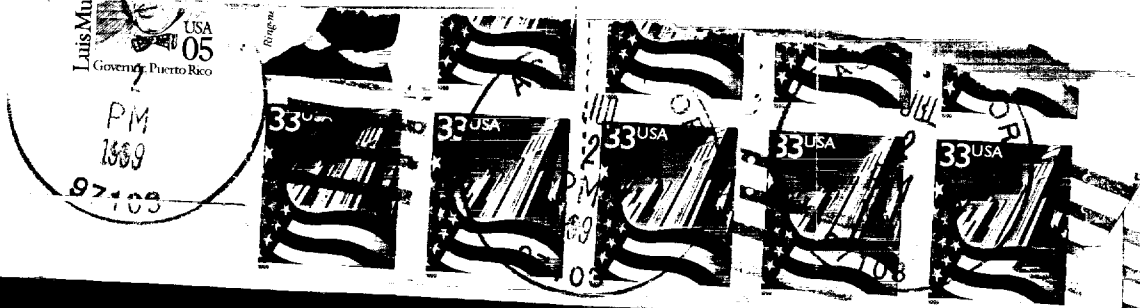
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